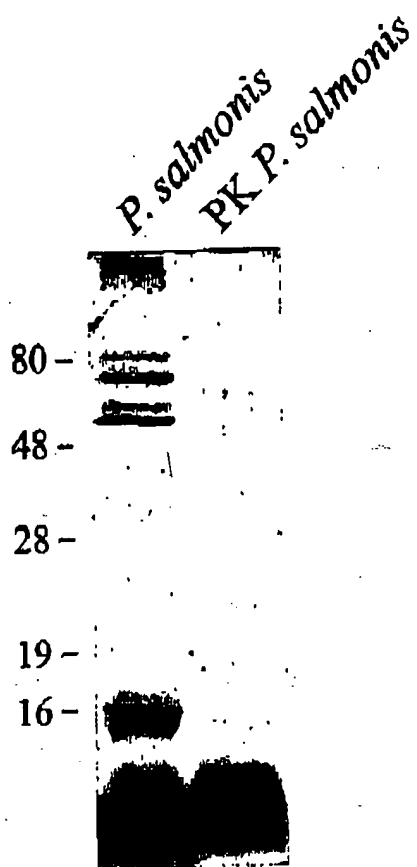


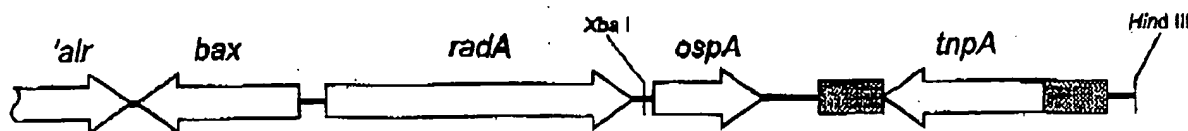
FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*



0057374-091500

FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAGGTAGTAGTCTAATTATTATCAGTGTGTTTTTGTAGTTGGCTGTGCCCA
ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCCGGTGTTCGTCGGCCAGCTGTTTGG
TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAAATTGGTTCTAAAATC
GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGAAAAGGTAAAAGCAGGGCAAG
TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTGAGCCAGTGCCTACTTACCAGCGTTA
CAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTTAGCAAAAAGGCGATGATTGCAGGGCAGAAG
CAAGAGATTTACGGCACTGCATGCCCGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

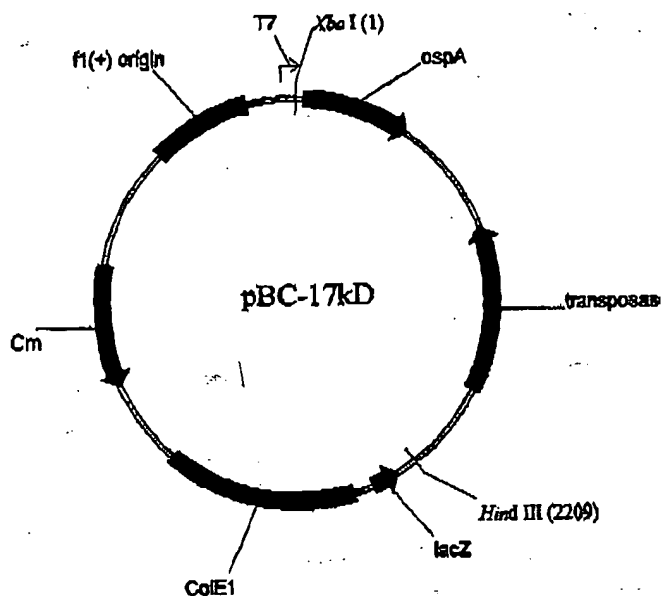
MNRGCLQGSSLIISVFLVGCQNFSRQEVGAATGAVVGGVAGQLFGKSGSRVAMAIGGAVLGGLIGSKI
GQSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQCYCREFOOKAMIAGQK
QEITYGTACROPDGRWQVISTEK

C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

	10	20	30	40
<i>P. salmonis</i> :	MNRGCLQGS	SLIISV---	SVFGCA--	QNFSDV
<i>R. prowazekii</i> :	MKLLSKIM	HALAAS	QDQNGSC	MNKQGT
	50	60	70	80
<i>P. salmonis</i> :	FGKSGSRV	AMAI	GGAVLG	GLIGSKI
<i>R. prowazekii</i> :	FGKSGSRV	AMAI	GGAVLG	GLIGSKI
	90	100	110	120
<i>P. salmonis</i> :	QSMDDQDK	IKLNQSL	KVKAGQ	VTRWRN
<i>R. prowazekii</i> :	QSMDDQDK	IKLNQSL	KVKAGQ	VTRWRN
	130	140	150	160
<i>P. salmonis</i> :	PDGNSYS	VEPVRT	YQRYNK	QERRQCY
<i>R. prowazekii</i> :	PDGNSYS	VEPVRT	YQRYNK	QERRQCY
	170	180	190	200
<i>P. salmonis</i> :	CREFOOK	AMIA	AGQKQ	
<i>R. prowazekii</i> :	CREFOOK	AMIA	AGQKQ	
	210	220	230	240
<i>P. salmonis</i> :	QEITYGT	ACROPD	GRWQVI	STEK
<i>R. prowazekii</i> :	QEITYGT	ACROPD	GRWQVI	STEK

FIGURE 3

A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.



B. Western blot analysis of OspA expression.

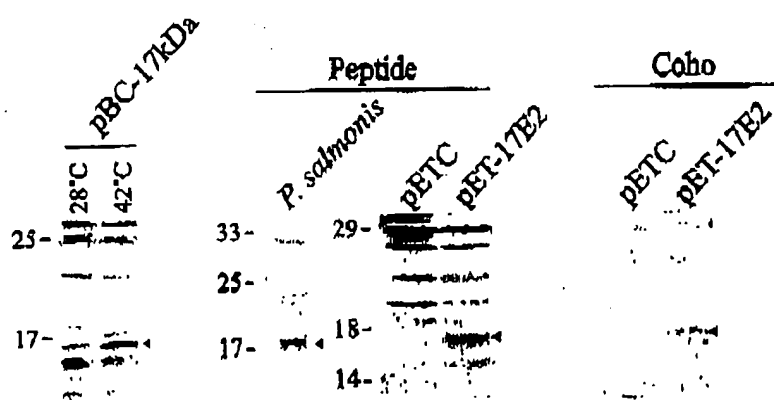
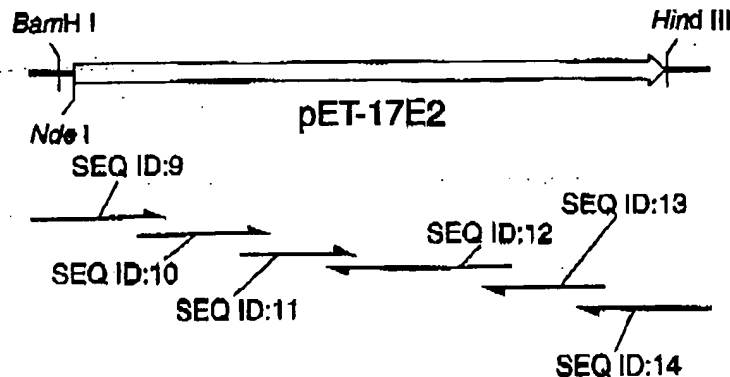


FIGURE 4.

A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.



B. Oligonucleotide #1 (SEQ ID:9)

CGCCAGGGTTTTCCAGTCAAGACGGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
ATTATCTCTGTTTTCTGGTGGGTTCGCCCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)

TGGGTTCGCCCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
CGGCCAGCTGTTCCGTAAAGGCTCTGGTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)

AAAGGCTCTGGTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)

GTTCCACAGAGTAGCTGTTACCGGTGTCCGGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTC
CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)

GGTGCCGTAGATTTCTGTTTTCTGACCTGCGATCATGGCTTTCTGCTGAAAATTCGCGGCAGTACTGCTGA
CGGCGTTTCTGTTTGTGTAAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)

CGTCCTCTCGTCCTGGTCCGAATTCAGATAAGCTTATTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
GGCTGACGGCACGCGGTGCCGTAGATTTCTGTTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGGTGGGTTCGCCCCAGAACT
TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTCCGTAA
AGGCTCTGGTCGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
CAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACAGTCTCTGGAAGAAAGTGAAAGCCGGCCAGGTTA
CTCGTTGGCGTAATCCGGACACCGGTAACAGTACTCTGTGGAACCGGTTCCGACCTACCAGCGTTACAA
CAAACAGGAACGCCGTGACGAGTACTGCCCGGAATTCAGCAGAAAGCCATGATCGCAGGTGAGAAACAG
GAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVSMAGGAVLGGLIGSKIG
QSMDOQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEFVRTYQRYNKQERRQOYCREFOQKAMIAGOKQ
EIIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

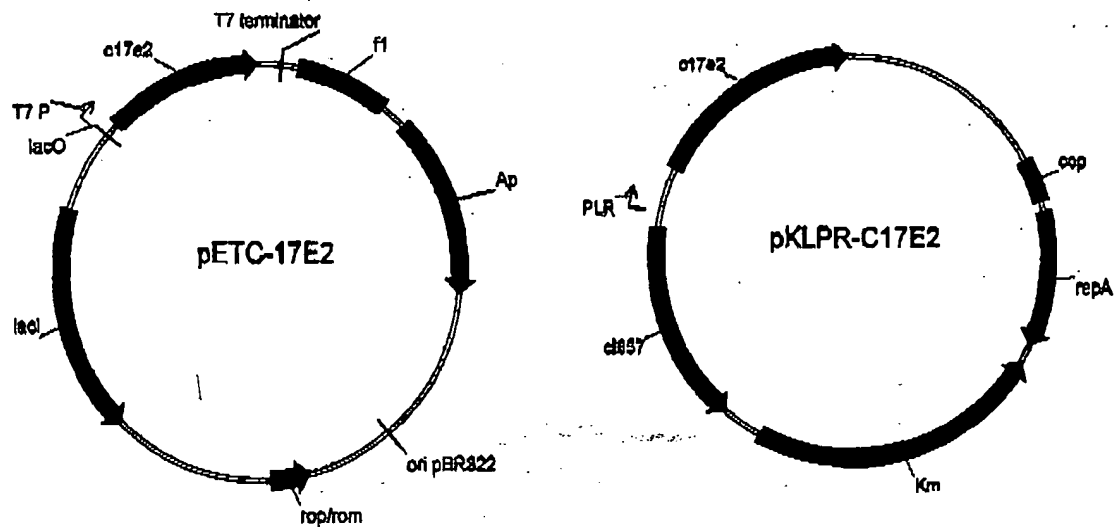
ATGTCAGTTGAATTCTACAACCTCTAACAAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTTAAATTTAAATGACGTAAAAGTTAGATATTATTACACRAAGTGATGGTAC
ACAAGGACAAACTTTCTGGTGTGACCATGCTGGTGCATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGTTGGGTTGCGCCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTT
GGTAAAGGCTCTGGTCTGTGTGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCACTCTCTGAAAAAGTGAAGCCGGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTGCGACCTACCAGCGT
TACAACAAACAGGAACGCCGTGAGCAGTACTGCCGCGAATTTGAGCAGAAAGCCATGATCGCAGGTGAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

MSVEFYNSNKSAAQTNSITPIIKITNTSDSDLNLDVKVRYYYTSDGTQGQTPWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDTYLDPSHMRGCLQGSSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKGSGRVSMAGGAVLGGLIGSKIGQSMDOQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEFVRTYQRY
YNKQERRQOYCREFOQKAMIAGOKQEIYGTACPQPDGRWQVISTEK

FIGURE 6

A. Expression vectors encoding the optimized *ospA* fusion constructs



B. SDS-PAGE analysis of C17E2 expression.

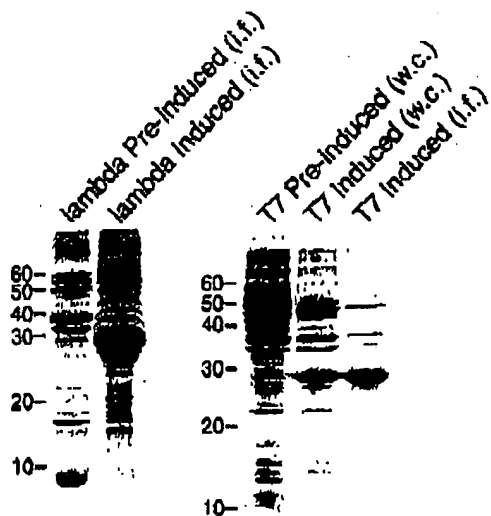


FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.

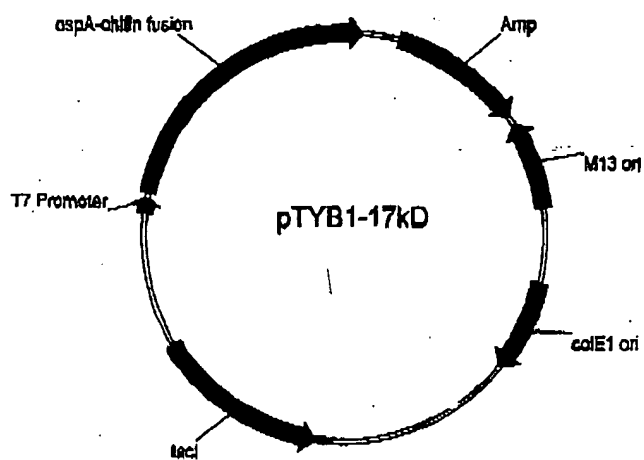
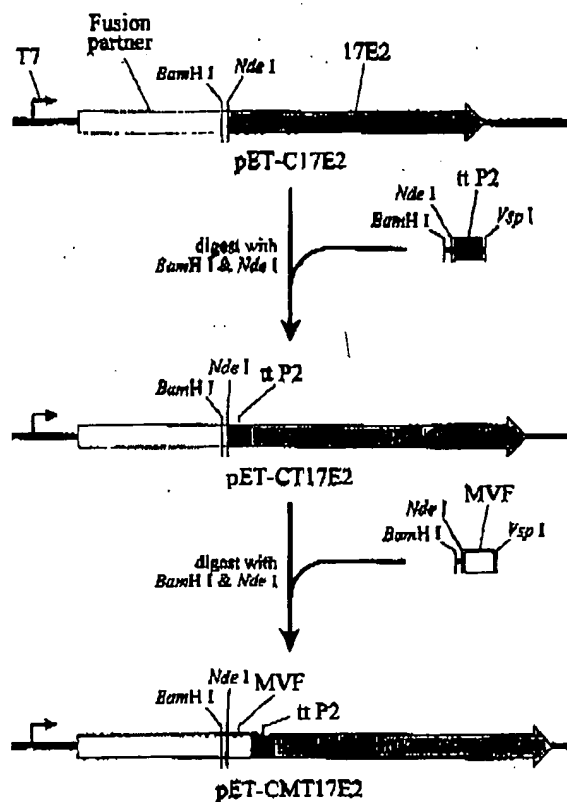


FIGURE 8

A. CLONING STRATEGY FOR OSPA TCE FUSION PROTEIN CONSTRUCTS.



B. (a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC
GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT
CGTCTGGAAGGCCTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVTVHRLEGV

FIGURE 9

Coho salmon antibody titres against OspA-fusion protein candidate vaccines.

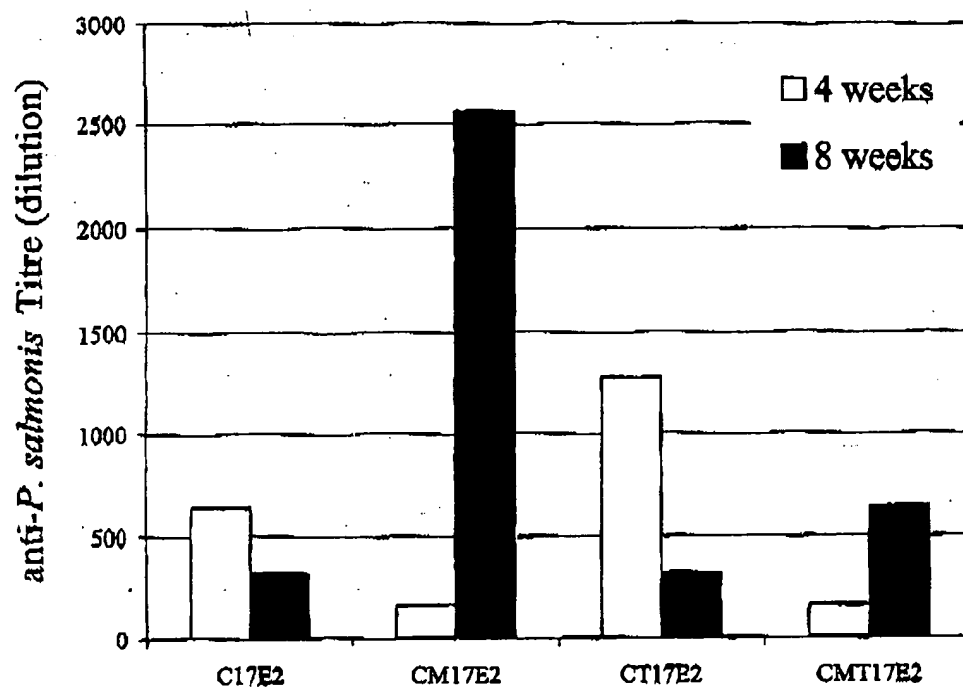


FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

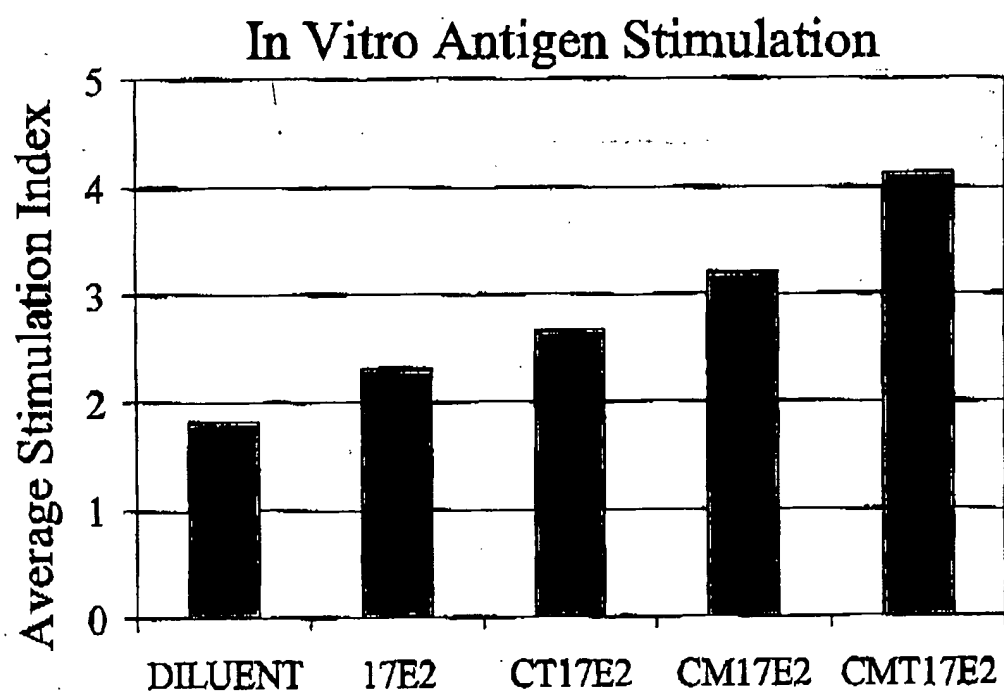


FIGURE 11

Vaccine trial in coho salmon of OspA fusion proteins.

